

The Council for Tobacco Research - U.S.A. Grant, "Studies of Nicotine Action Upon Memory Consolidation."

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PROGRESS REPORT

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STUDIES OF NICOTINE ACTION UPON MEMORY CONSOLIDATION

Within the past 17 months, or since the submission of a previous progress report for this research project (January 16, 1973 - Aug. 1, 1974), the research has been specifically concerned with the interaction of putative neurotransmitters and the central effects of nicotine and several of its biologically active metabolites. Changes in brain serotonin (5-hydroxytryptamine) metabolism have, in previous research, been established in conjunction with several amnesic agents and/or events, several of which appear to be temporally contiguous with the amnesic associated inhibition of cerebral protein synthesis. Such effects, particularly as brought about experimentally through the administration of electroconvulsive shock (ECS) have been antagonized or blocked through the administration, prior to administration of the amnesia stimulus of nicotine. These effects have been documented in previous publications from our laboratory. We have also demonstrated that several metabolites of nicotine, namely cotinine and 3-pyridylacetic acid, are capable of antagonizing the amnesic effects brought about by post-training ECS and similarly alter the effects of such ECS upon brain 5-HT metabolites. These effects have also been shown to be time-dependent to the extent that 45 minutes appears to be optimal following nicotine injection, after which the amnesic properties of ECS are antagonized, whereas 15 minutes after treatment, the effects appear to be potentiated. In Figure 1, these behavioral data have been summarized for nicotine and several of its derivatives.

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As may be observed in Figure 2, the ratio of 5-HT and its metabolites in brain, 5-hydroxyindoleacetic acid (5-HIAA), is regionally modified following nicotine treatment and this effect is certainly consistent with observations that have been summarized in Figure 3. They show that nicotine administration procedures reduced turnover rate and increased turnover time for this brain amine.

The regional concentration of 5-HT and its metabolite in several regions of the mouse brain at those times at which different potential or antagonistic effects of nicotine have been shown in the amnesic paradigm, summarized in Table 1, from which it may be noted that the time difference accounts to a considerable extent for the change in regional 5-HT metabolism.

The question of the cellular specificity of this change has also been explored for different regions of the mouse brain that have been fractionated to yield cell pools enriched with their neuronal cell bodies or glia. These data, summarized in Figure 4 indicate that the neuronal as well as glial pools of the amine have been increased as a consequence of nicotine treatment. The possible bearing of this finding to the regional effects of nicotine and several related compounds to its interaction with such amnesic stimuli as ECS may be observed in Table 2, as compared with saline control treatment, significant changes in several brain regions apparently participated in the mediation of nicotine effects, have been documented.

The neurochemical consequences of cerebral electroshock have been explored and analyzed in a recent monograph wherein the interaction with nicotine, which may be relative to memory consolidation phenomenon, have been explored.

The age-dependent nature of amnesic effects produced by several experimental conditions and their relationship to the process of cerebral protein synthesis

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and the effects thereupon of nicotine, have also been considered. From these experiments, the concept of a "Critical Age" phenomena has emerged, such that in the 17-day-old CF-1S strain mouse, an analog of the central effects of nicotine observed in older mice, behaviorally as well as biochemically, has emerged. It was shown in this context that the intracranial administration of 5-hydroxytryptamine (5-HT) was capable of inducing an age-dependent amnesic effect, as well as an age-dependent effect upon the synthesis of cerebral protein.

The cholinergic effects of nicotine, although well-known, were further investigated on a subcellular level to specifically examine changes in the size of various cholinergic pools in the cerebral cortex. These results have indicated that administration of nicotine leads to a decrease in the acetylcholine content of both bound and vesicular storage pools without any alteration in the concentration of free acetylcholine.

The effects of nicotine were also examined in relation to the differential effect of this compound among environmentally conferred differences in learning ability. These previously published data have indicated that task-specific facilitative effects of nicotine exert different effects both behaviorally and metabolically depending upon the endogenous behavioral and metabolic baseline upon which they are superimposed. Such a model has been derived utilizing differentially housed mice. In animals treated with radioactive nicotine, it was found that isolated animals showed a greater brain uptake, for several regions of nicotine, as compared to group housed mice, where the only appreciable uptake occurred in the basal ganglia; the latter change, however, was markedly below the uptake of nicotine shown by isolated animals. Nicotine metabolites, specifically cotinine, were also found to be present in considerably greater quantities in several regions of the brain of isolated animals. This finding would be consistent with our previous suggestion that the facilitative effect of nicotine treatment upon the learning

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ability of behaviorally retarded mice (as result from early isolation housing) may be dependent upon a biologically active metabolite of nicotine, rather than to nicotine per se. A likely candidate for such a metabolite is cotinine, which we have observed to exert potent biochemical and behavioral effects in earlier studies.

was capable of inducing an age-dependent amnesia effect, as well as an age-dependent effect.

In several studies we have also examined the regional differences in cerebral protein synthesis exerted by nicotine at several subcellular sites. In this regard, we have conclusively established that microsomal protein synthesis in mouse cerebral cortex is significantly augmented as a consequence of nicotine treatment, and further that the protein synthesis inhibition which may be environmentally produced by the stress of isolation housing may be completely reversed at the microsomal level by treatment with nicotine. In this respect, preliminary studies of mitochondrial proteins from mouse cerebral cortex have indicated temporal qualitative and quantitative differences in the effects of nicotine. It is our purpose to further examine these changes and characterize them with respect to some of the metabolites of this compound.

In a final series of studies, progress of which may be indicated at this point, it was shown that an experimentally-induced retrograde amnesia effected in mice with electroconvulsive shock could be antagonized or potentiated depending upon the interval of time between nicotine treatment and the post-conditioning amnesic event. In this regard, it was shown that conditioned response retention was reduced below control levels if nicotine treatment was given 15 minutes prior to ECS, whereas if such treatment was effected 45 minutes prior to treatment, approximately 80% of the animals showed antagonism toward the amnesic effect.

These results again are consistent with our earlier findings and provide further support for the general working hypothesis that the role of nicotine in memory

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consolidation is to provide temporally related facilitative effects as are dependent upon aminergic and protein synthetic interactions in the central nervous system. A likely candidate for such a metabolite is nicotine, which we have observed to exert potent sleep-inducing and memory-consolidating effects.

Currently under development are a series of procedures designed to provide for in vitro assessment of in vivo effects of nicotine and amnesic changes in relationship to the distribution of cerebral amines and the synthesis of cerebral proteins. These studies have focused upon microsomal systems, isolated from several regions of the mouse brain and have indicated that several of the parameters by which nicotine metabolism, central distribution of nicotine and/or metabolites and behavioral effects of nicotine and/or metabolite specific memory consolidation phenomenon have been regulated, such as differential housing, provide for differences observed utilizing in vitro protein synthesis methods.

More specifically in this context, it has been observed that in vivo depletion of 5-hydroxytryptamine (5-HT), differential housing, or in vivo treatment, acutely or chronically with nicotine, can significantly alter the rate of microsomal protein synthesis measured in vitro, or modify the inhibitory effect of 5-HT upon such protein synthesis.

The significance of memory consolidation and the model proposed for one facet of its operation relates to other conditions to which memory-like mechanisms may, by analogy, be cited. For example, the very interesting work of D. Clouet has indicated that rats that developed an addictive state in narcotic drugs and are subject to withdrawl symptoms subsequent to drug abstinence do both things in this spectrum if treated with anti-biotics such as puromycin. If such a regimen is carried out, addiction does not develop, dose habituation does not occur, and there is an absence of withdrawl signs. This may perhaps illustrate one form of memory in the sense that a state-dependent physiology phenomenon

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may be obviated for the synthesis of proteins with which it must be apparently induced has been inhibited. Another example may be found in the area of state-dependent learning, wherein animals treated with barbiturates such as pentobarbital or phenobarbital are capable of learning responses while under the influence of such barbiturates; further, such responses are manifest when tested in the non-barbiturate state. When barbiturates are again administered to such animals, such may be elicited in the barbiturate state. A parallel between these latter experiments and animal tissue inhibition in the study in progress is that such barbiturates do elevate brain 5-HT content and 5-HT level drops following barbiturate withdrawal. This, based upon our previous data, shows a relationship between 5-HT level and protein synthesis; the analogy may be extended further to suggest that inhibition of protein synthesis is 5-HT dependent and thereby state dependency is a related event to protein synthesis dependency; both are 5-HT metabolites.

With regard to our more recent findings concerning short chain fatty acids, it might be pointed out that the particular relevance of these substances to the present project and to the hypothesis to be tested in the proposed research is that these compounds are produced in excess with a variety of hepatic dysfunctional states, such as cirrhosis, hepatolenticular degeneration, coma, and drug induced toxicity. Experimental mediation of coma by administration of short chain fatty acids has been shown with such agents as butyric acid, valeric acid, and octanoic acid in doses ranging from  $10\mu\text{M}$  per kg to  $25\mu\text{M}$  per kg. It was further apparent that doses capable of rapidly inducing coma, of brief duration, were also capable of mediating a retrograde amnesia for a passive avoidance conditioned response increasing the concentration of brain 5-HT leading to a marked reduction in the synthesis of proteins in several regions of the mouse brain. These studies have further provided a base line upon which subsequent investigations are contemplated.

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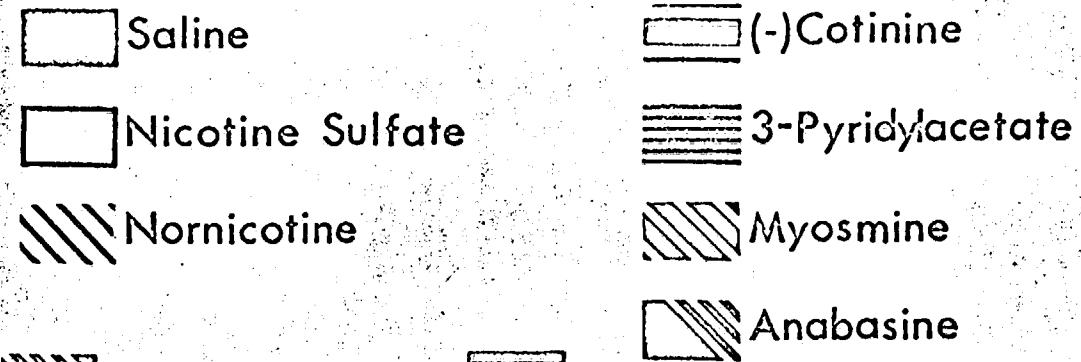
Per Cent Incidence of Conditioned Response Retention

100  
80  
60  
40  
20

15 Min. P-I Training

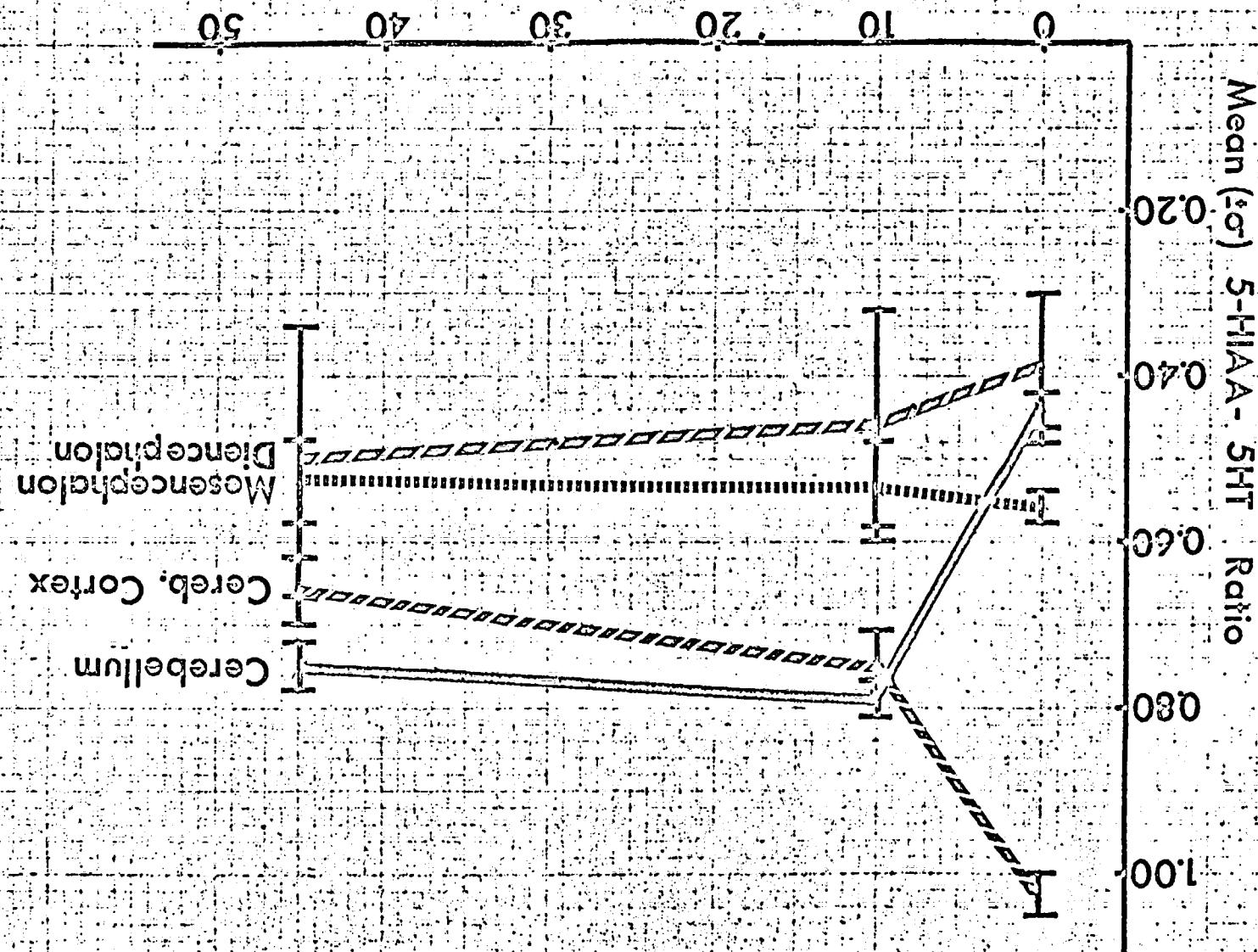
45 Min. P-I Training

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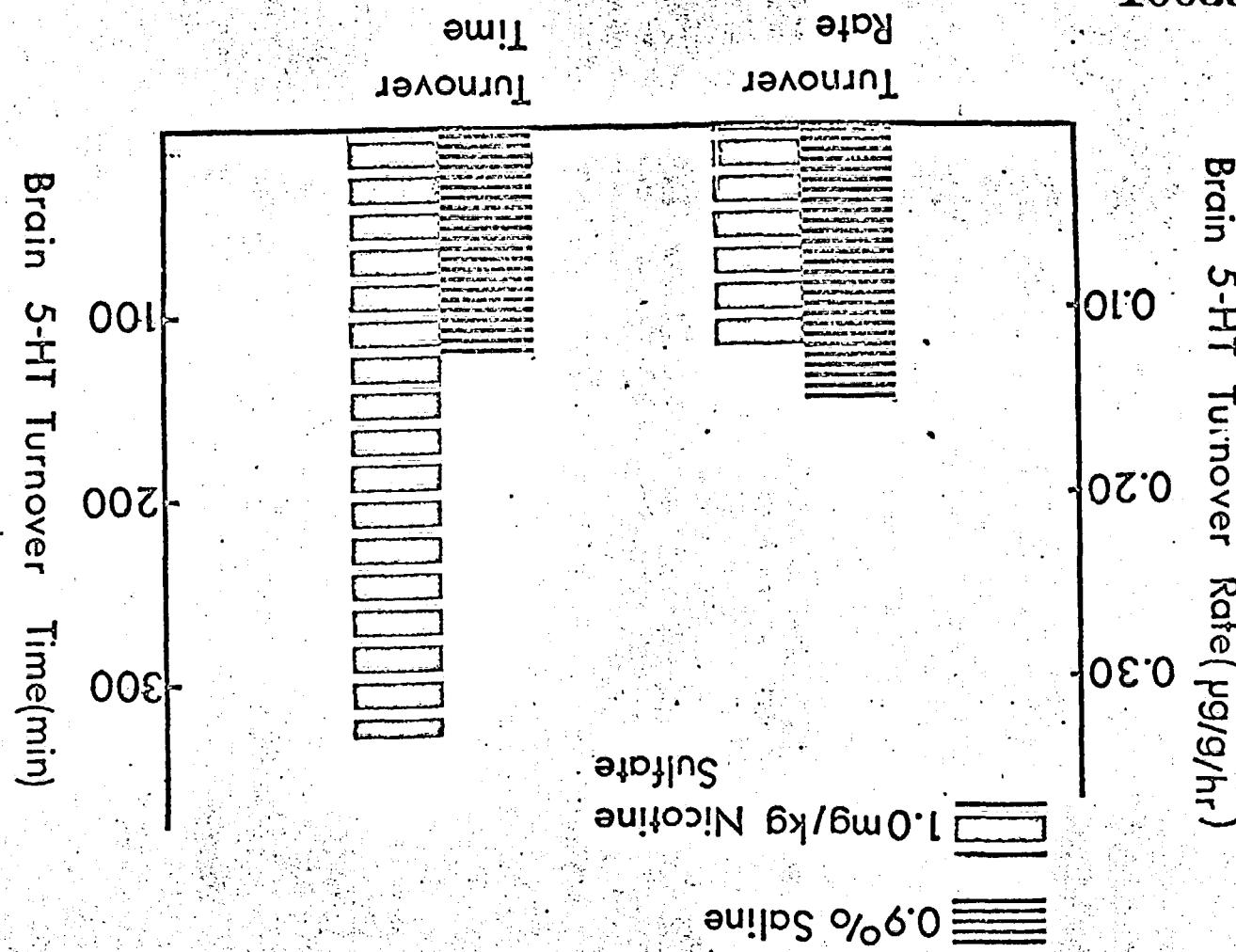


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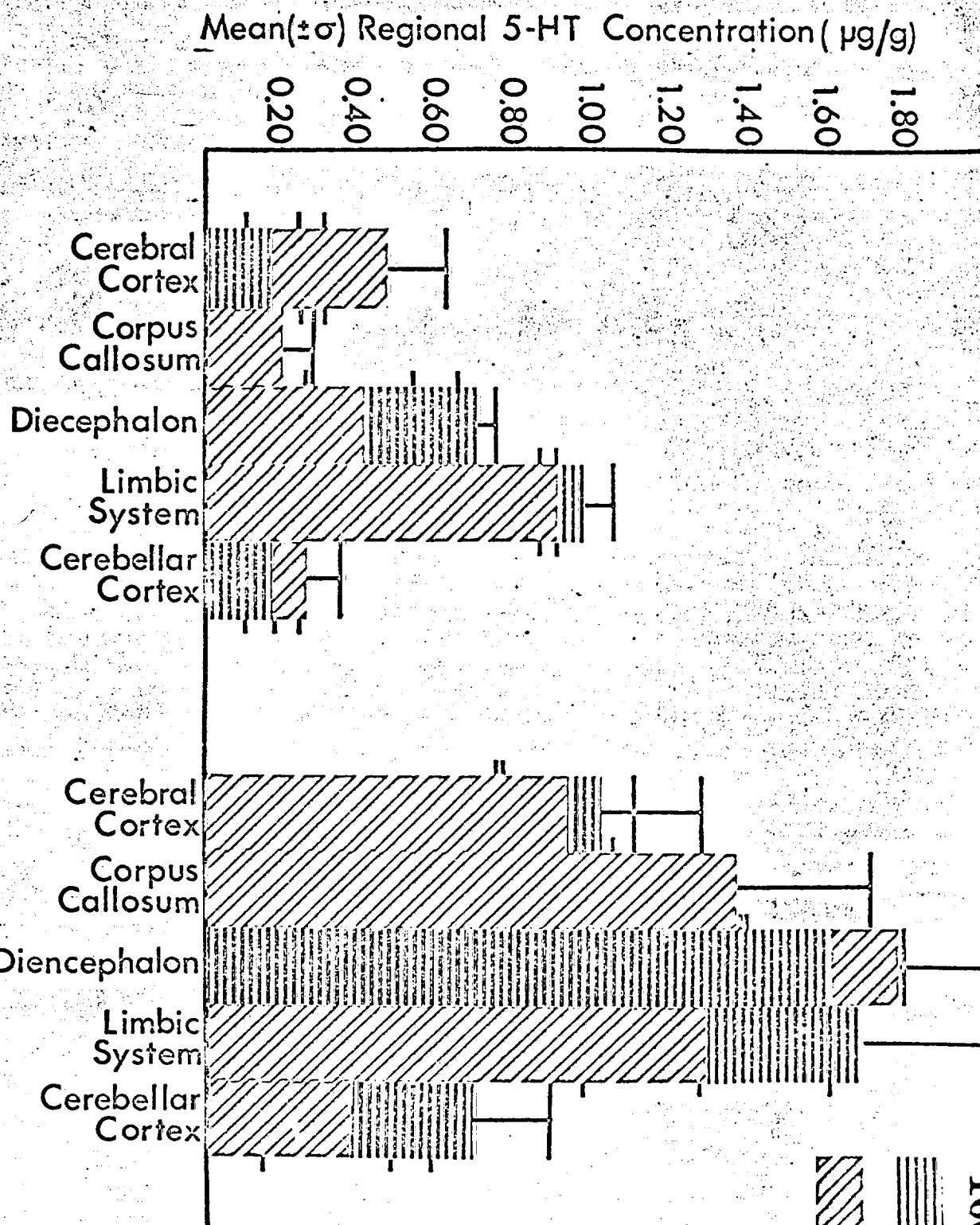
Time After Nicotine Treatment



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Saline 0.9%



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Glia-Enriched

Neuronally-Enriched

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\* Concentrations expressed as  $\mu\text{g/g}$ .

BRAIN AREA	SALINE		NICOTINE		COTININE		SALINE		NICOTINE		COTININE	
	15	45	15	45	15	45	15	45	15	45	15	45
CEREBRAL CORTEX: S-HIAA	0.38 (0.06)	0.42 (0.01)	0.33 (0.04)	0.39 (0.04)	0.43 (0.05)	0.12 (0.02)	0.66 (0.04)	0.28 (0.03)	0.52 (0.19)	0.55 (0.06)	0.33 (0.01)	0.42 (0.06)
MESENCEPHALON: S-HIAA	0.33 (0.04)	0.82 (0.02)	0.83 (0.05)	0.33 (0.04)	0.36 (0.02)	0.45 (0.02)	0.36 (0.04)	0.21 (0.02)	0.33 (0.05)	0.45 (0.02)	0.21 (0.04)	0.33 (0.05)
MESENCEPHALON: S-HT	0.21 (0.02)	0.45 (0.02)	0.36 (0.04)	0.18 (0.04)	0.18 (0.03)	0.64 (0.06)	0.35 (0.06)	1.00 (0.12)	0.33 (0.06)	0.33 (0.05)	0.33 (0.05)	0.21 (0.02)
DIENCEPHALON: S-HIAA	0.33 (0.04)	0.96 (0.20)	0.56 (0.04)	0.56 (0.09)	0.29 (0.04)	0.33 (0.05)	0.14 (0.03)	0.90 (0.15)	0.45 (0.18)	1.07 (0.11)	0.28 (0.05)	0.45 (0.03)

MEAN ( $\pm$ ) REGIONAL CONCENTRATION OF S-HYDROXYTRYPTAMINE (S-HT) AND S-HYDROXYINDOLEACETIC ACID (S-HIAA) FOLLOWING SALINE-OR DRUG-TREATMENT\*

TABLE I.

MEAN ( $\pm \sigma$ ) REGIONAL CONCENTRATION OF BRAIN 5-HYDROXYTRYPTAMINE (5-HT) AND 5-HYDROXYINDOLEACETIC ACID (5-HIAA)  
IN DRUG-TREATED MICE GIVEN ELECTROCONVULSIVE SHOCK (ECS) OR SHAM-ELECTROCONVULSIVE SHOCK (ECS).

TREATMENT AND REGION SAMPLED	POST-INJECTION (P.I.) CONDITION							
	ECS 15' P.I.		ECS 45' P.I.		ECS 15' P.I.		ECS 45' P.I.	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
<b>NORNICOTINE</b>								
Cerebral Cortex	0.33 (0.03)	0.15 * (0.01)	0.28 * (0.06)	0.15 * (0.03)	0.33 (0.02)	1.79 (0.16)	0.67 (0.12)	0.86 (0.14)
Olfactory Bulbs	0.04 (0.01)	0.02 (0.00)	0.02 (0.00)	0.02 (0.00)	0.03 (0.01)	0.04 (0.01)	0.08 (0.01)	0.02 (0.01)
Mesencephalon	0.44 * (0.08)	0.95 * (0.10)	0.33 * (0.04)	0.21 * (0.03)	0.33 (0.02)	1.89 (0.18)	0.83 (0.10)	0.57 (0.06)
Diencephalon	0.44 (0.07)	1.35 (0.15)	0.33 * (0.03)	0.12 * (0.02)	0.28 (0.01)	1.94 (0.17)	0.78 (0.09)	1.00 (0.13)
Cerebellum	0.04 (0.01)	0.03 (0.01)	0.03 (0.00)	0.01 (0.00)	0.03 (0.01)	0.01 (0.00)	0.06 (0.01)	0.05 (0.01)
<b>ANABASINE</b>								
Cerebral Cortex	0.38 * (0.02)	0.24 * (0.02)	0.33 (0.05)	1.57 * (0.18)	1.83 (0.16)	1.00 (0.17)	0.40 (0.12)	0.71 (0.16)
Olfactory Bulbs	0.03 (0.01)	0.02 (0.00)	0.03 (0.01)	0.06 (0.01)	0.08 (0.02)	0.06 (0.02)	0.03 (0.01)	0.02 (0.01)
Mesencephalon	0.28 * (0.04)	0.24 * (0.01)	0.39 (0.06)	0.43 * (0.04)	0.83 (0.09)	1.28 (0.16)	0.39 (0.10)	0.18 (0.04)
Diencephalon	0.28 * (0.04)	0.20 * (0.06)	0.39 (0.07)	0.57 (0.04)	0.78 (0.06)	1.00 (0.19)	0.44 (0.11)	0.24 (0.03)
Cerebellum	0.03 (0.01)	0.03 (0.01)	0.04 (0.01)	0.03 (0.01)	0.06 (0.02)	0.02 (0.00)	0.01 (0.00)	0.02 (0.01)
<b>3-PYRIDYLACETIC ACID</b>								
Cerebral Cortex	0.39 * (0.09)	0.36 (0.03)	0.33 * (0.03)	0.36 (0.04)	0.44 (0.05)	0.36 (0.04)	1.39 (0.16)	0.86 (0.14)
Olfactory Bulbs	0.04 (0.01)	0.03 (0.01)	0.05 (0.01)	0.04 (0.01)	0.05 (0.02)	0.03 (0.01)	0.06 (0.01)	0.02 (0.00)
Mesencephalon	0.44 (0.06)	0.85 * (0.05)	0.39 * (0.04)	0.43 (0.06)	0.44 (0.04)	0.36 (0.03)	0.56 (0.07)	0.36 (0.04)
Diencephalon	0.39 * (0.03)	1.00 * (0.09)	0.33 * (0.03)	0.43 * (0.05)	0.44 (0.06)	0.36 (0.04)	0.50 (0.04)	0.36 (0.03)
Cerebellum	0.04 (0.01)	0.05 (0.01)	0.05 (0.01)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.06 (0.02)	0.02 (0.01)

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TREATMENT AND REGION SAMPLED	POST-INJECTION (P.I.) CONDITION							
	ECS 15' P.I.		ECS 45' P.I.		ECS 15' P.I.		ECS 45' P.I.	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
<u>(-) COTININE</u>								
Cerebral Cortex	0.39 (0.04)	0.36* (0.03)	0.33 (0.04)	0.15* (0.02)	0.33 (0.04)	0.43 (0.05)	0.28 (0.03)	0.36 (0.04)
Olfactory Bulbs	0.04 (0.01)	0.03 (0.00)	0.03 (0.00)	0.02 (0.00)	0.03 (0.01)	0.05 (0.01)	0.03 (0.00)	0.05 (0.01)
Mesencephalon	0.44* (0.07)	0.86* (0.07)	0.39 (0.03)	0.15 (0.03)	0.83 (0.05)	0.36 (0.04)	0.33 (0.05)	1.00 (0.12)
Diencephalon	0.44 (0.05)	0.29 (0.04)	0.33 (0.02)	0.12 (0.02)	0.56 (0.04)	0.29 (0.04)	0.28 (0.05)	1.07 (0.11)
Cerebellum	0.04 (0.01)	0.05 (0.01)	0.03 (0.01)	0.01 (0.00)	0.06 (0.01)	0.05 (0.02)	0.03 (0.01)	0.03 (0.01)
<u>MYOSMINE</u>								
Cerebral Cortex	0.39* (0.06)	0.36* (0.04)	0.28* (0.04)	0.86* (0.14)	0.90 (0.19)	0.15 (0.02)	0.44 (0.09)	0.15 (0.03)
Olfactory Bulbs	0.02 (0.00)	0.04 (0.01)	0.03 (0.01)	0.04 (0.01)	0.06 (0.02)	0.01 (0.00)	0.03 (0.01)	0.01 (0.01)
Mesencephalon	0.44* (0.07)	0.31* (0.05)	0.28* (0.06)	0.57* (0.09)	0.56 (0.14)	0.15 (0.03)	0.38 (0.06)	0.15 (0.04)
Diencephalon	0.39* (0.05)	0.43* (0.06)	0.28 (0.05)	0.57* (0.11)	0.61 (0.10)	0.12 (0.03)	0.28 (0.04)	0.15 (0.03)
Cerebellum	0.04 (0.01)	0.03 (0.01)	0.03 (0.01)	0.04 (0.01)	0.00 (0.00)	0.01 (0.00)	0.03 (0.01)	0.01 (0.00)
<u>SALINE</u>								
Cerebral Cortex	0.38 (0.06)	0.12 (0.03)	0.33 (0.04)	0.15 (0.03)	0.38 (0.06)	1.52 (0.19)	0.39 (0.04)	0.12 (0.02)
Olfactory Bulbs	0.03 (0.01)	0.01 (0.00)	0.03 (0.01)	0.01 (0.00)	0.03 (0.01)	0.01 (0.00)	0.07 (0.02)	0.02 (0.01)
Mesencephalon	0.33 (0.04)	0.12 (0.02)	0.33 (0.05)	0.15 (0.03)	0.33 (0.04)	0.21 (0.02)	0.33 (0.04)	0.18 (0.03)
Diencephalon	0.39* (0.04)	0.15 (0.03)	0.33 (0.06)	0.15 (0.04)	0.33 (0.04)	0.15 (0.03)	0.33 (0.05)	0.41 (0.03)
Cerebellum	0.03 (0.01)	0.03 (0.01)	0.03 (0.01)	0.02 (0.00)	0.03 (0.01)	0.02 (0.01)	0.03 (0.01)	0.02 (0.00)

\*p<.01

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